Tropical Biomedicine 34(3): 550–555 (2017)

Survey of the prevalence of *Toxocara cati* in stray cats in Isfahan city, Iran by PCR method

Torkan, S.[1], Ghandehari-Alavijeh, M.R.[2] and Khamesipour, F.[3,4*]
[1]Department of Small Animal Internal Medicine, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[2]Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[3]Cellular and Molecular Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran
[4]Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
[*]Corresponding author e-mail: Dr_Faham@yahoo.com; F.Khamesipour@iaushk.ac.ir
Received 26 September 2016; received in revised form 10 May 2017; accepted 14 May 2017

Abstract. Toxocariasis is a parasitic zoonosis with worldwide distribution that affects both cats and dogs. This parasite is one of the factors contributing to visceral larva migrans and ocular larva migrans in humans. Therefore, it is crucial to gain a good understanding of how cats are infected by *T. cati*, so as to prevent citizens from infection. This study aimed to examine the prevalence of *T. cati* in Isfahan. In 2014-2015, a total of 147 feacal samples were collected from urban stray cats. The felid feaces were analyzed through PCR test and 26 cats (17.7%) were diagnosed with *T. cati* gene. Sixteen cats (10.88%) were female and 10 cats (6.8%) were male. In terms of age group, 9 cats (6.12%) were adult while 17 cats (11.56%) were immature. Unlike previous studies there was no significant relationship between age/sex and prevalence of the parasite. Since there is a close link between humans and cats with greater risk of transmitting common diseases particularly in children, it is critical to raise public awareness about the disease and advise adults to be more health-conscious outdoors.

INTRODUCTION

Gastrointestinal parasites are the main causes of morbidity in domestic cats (Yang & Liang, 2015). The prevalence of intestinal parasites is affected by several factors such as geographical region; presence of veterinary care; habits of the local animal populations; season of the year and the cat population composition. Several epidemiological surveillance studies reported that stray cats present high prevalence of parasites (Calvete *et al*., 1998; McColm & Hutchison, 1980; Niak, 1972; Nichol *et al*., 1981). Toxocariasis, caused by infection with larvae of *Toxocara canis* (*T. canis*), and to a lesser extent by *Toxocara cati* (*T. cati*), manifests in humans in a range of clinical syndromes. These include visceral and ocular larva migrans, neurotoxocariosis and covert or common toxocariosis (Macpherson, 2013).

T. cati is a common gastrointestinal nematode in cats worldwide, which not only infects stray cats but can also cause human toxocariosis (Dubinsky, 1999) and responsible for visceral larvae migrans (VLM) and ocular larva migrans (OLM) in humans (Fisher, 2003; Lee *et al*., 2010). The close association between cats and humans is responsible for the high endemicity of some of these zoonotic diseases (Overgaauw, 1997).

Transmission of certain helminth parasites of carnivores to domestic animals and human causes economic problems and public health hazards (Dalimi & Mobedi, 1992). *T. cati* is mostly prevalent throughout tropical, subtropical and temperate regions (Mizgajska-Wiktor

& Uga, 2006), where visceral larva migrans is one of the most important parasitic disease of man transmitted by carnivores in Iran (Dalimi & Mobedi, 1992). Gates and Nolan (2009) and have been carried out in order to identify the significance of feral cats as potential reservoirs of infection (Calvete *et al*., 1998).

There have been few detailed and comprehensive studies on the prevalence of parasites in stray cats (Zibaei *et al*., 2007; Arabali & Hooshyar, 2009; Kreceka *et al*., 2010) and there is little information on the prevalence of *T. cati* parasitic infection in stray cats using molecular techniques in Isfahan, Iran. Recently, polymerase chain reaction (PCR) have been revealed to be valuable and rapid tool for diagnosis of animal and human diseases (Khamesipour *et al*., 2014b; Nekoei *et al*., 2015; Shakerian *et al*., 2016; Tajbakhsh *et al*., 2016; Hosseini *et al*., 2017). This study allowed generation of information on the distribution of parasitism and its significance to the health of humans and animals inhabiting the area under study. Therefore, the main objective of this study was to determine the prevalence of *T. cati* parasites in stray cats in Isfahan city, Iran.

MATERIALS AND METHODS

Study Area, Design, and Population

In 2014-2015, a total of 147 feacal samples (adult: 90 and immature: 57) (male: 72 and female: 75) were collected from open public spaces in five regions of Isfahan city (north, south, east, west, and central) in Iran. The sampling procedure required no specific permission from the city and the study did not involve protected species. Approximately 50 g of cat feaces was collected and the residual portions of the samples were hygienically discarded.

Sample Collection and Fecal Analysis

Fecal samples were put in plastic bags and stored at 4°C until processing within 24 h. Isolation of the eggs of *Toxocara* spp. from each sample was performed by the sucrose flotation method as previously described (Rai *et al*., 2000). Fecal samples were suspended in distilled water, centrifuged at 1000 rpm for 5 min, and approximately 30 ml distilled water was added to the sediment. The suspensions were layered with 15 ml of sucrose (Merck, Germany), with specific gravity of 1.40, and centrifuged at 800×g for 5 min. The upper layer of the liquid was separated and centrifuged at 1000×g for 5 min. The sediment was then resuspended in 50 ml distilled water and centrifuged at 5000 rpm for 5 min. Finally, the samples were washed twice with distilled water and sediments were examined under light microscopy (10× and 40×).

DNA Isolation and PCR

Genomic DNA from the eggs of *Toxocara* spp. was extracted using the CinnaGen DNA Extraction Kit (CinnaGen, Tehran, Iran) according to the manufacturer's instructions. The samples were first subjected to three freeze-thaw cycles, and proteinase K digestion was performed overnight (~16 h) as suggested by Borecka & Gawor (2008). Species-specific oligonucleotide primers were selected from internal transcribed spacer 2 (ITS2) gene sequences that were previously described as *NC2* (*5'-TAGTTTCTTTTCCTCCGCT-3'*) and *Tcat1* (*5'-GGAGAAGTAAACTC-3'*) for *T. cati* (Khademvatan *et al*., 2013).

PCR amplification was performed using 50 µL of reaction mixture containing 250 µM of each deoxynucleotide, 100 pmol of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 9), 3 mM $MgCl_2$, 10% dimethyl sulfoxide (DMSO, Sigma), 2 U of Taq DNA polymerase (Fermentas), and 10-15 ng of template DNA. The PCR reaction was conducted using a thermal cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) and the amplification cycle consisted of an initial cycle at 94°C for 30 s; followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s; and a final extension cycle at 72°C for 10 min. Finally, 20 µL of PCR products were run on a 1.8% agarose gel containing ethidium bromide in 1× Tris-

borate EDTA buffer along with 1444–80 bp DNA ladder (Fermentas).

Statistical Analysis

Statistical analysis was performed using SPSS 19.0 software. Chi-square test was used to test differences between groups at $P \leq 0.05$.

RESULTS

Of the 147 stray cats used in this study, 90 were adult and 57 were immature and also 72 were male and 75 were female (Table 2).

PCR results showed that of the total 147 stray cats, 26 (17.7%) were found to be infected with *T. cati* (Table 1). Therefore, the fecal parasite infections indicated that the overall infection rate was 17.7%.

Of the 26 cats diagnosed with *T. cati* gene, 16 (10.88%) were female and 10 (6.8%) were male. In terms of age group, 9 cats (6.12%) were adult while 17 (11.56%) were immature (Table 2). In this study there was no significant relationship between age/sex and prevalence of the parasite ($P>0.05$).

Table 1. *Toxocara cati* infected stray cats according to age and sex in Isfahan, Iran

Variable	No. of cats examined	Cats infected no. (%)
Age		
Adult	90	9 (6.12)
Immature	57	17 (11.56)
Sex		
Male	72	10 (6.80)
Female	75	16 (10.88)
Total	147	26 (17.68)

Table 2. *Toxocara cati* infection rate in stray cats in Isfahan, Iran

Animal	Number	Percent
Infected stray cats	26	17.7
Non-infected stray cats	121	82.3
Total	147	100

DISCUSSION

T. cati is one of the most widespread public health and economically important zoonotic parasitic infections humans share with dogs, cats and wild canids (Macpherson, 2013). Amongst the zoonotic agents transmitted by cats, *Toxoplasma gondii* and *T. cati* are among the most important feline gastrointestinal parasites (Robertson & Thompson 2002; Khamesipour *et al*., 2014a).

The fecal parasite infections in the current study indicated that the overall infection rate of *T. cati* was 17.7% which is relatively lower in comparison to the prevalence encountered in Denmark (79%) (Haralampidis, 1977), in Spain (55%) (Calvete *et al*., 1998), in Greece (67%) (Haralampidis, 1977) and in England (53%) (Nichol *et al*., 1981).

One study in Turkey showed that the prevalence of *T. cati* in cats was 62.5% (Yaman *et al*., 2006), whereas, another study in Estonia reported an incidence of 48.2% in adult cats (Talvik *et al*., 2006). There are also several reports from other countries in the world which reported prevalence of 3-85% (Overgaauw, 1997; Calvete *et al*., 1998; Barutzki & Schaper, 2003; Sommerfelt *et al*., 2006; Palmer *et al*., 2008; Mircean *et al*., 2010) with higher prevalence reported in kittens (Visco *et al*., 1978).

On the other hand, the prevalence of helminthic infection of gastrointestinal tract of 98.5% have been recorded in Urban areas in Isfahan, Iran by Jamshidi *et al*. (2002) and 29% in Tehran, Iran by Bahadori *et al*. (2004). The findings confirm a trend in Iran of a decrease in the prevalence of helminthes parasites in cats. It is evident that *T. cati* parasites are now the common parasites affecting stray cats in Isfahan, Iran, with prevalence considerably lower than in previous studies. This is important

in respect to the distribution of *T. cati* eggs in the environment, because every female *Toxocara* produces about 200000 eggs per day (Glickman & Schantz, 1981) that can be transmitted to human and paratenic hosts after development in the soil.

In our study, among 26 cats diagnosed with *T. cati* gene, 10.88% were female and 6.8% were male. A similar finding was reported by Malloy & Embil (1978).

In the current study, sex seemed to have no effect on prevalence of parasitism. Therefore, the present study confirms the findings of other studies (Jamshidi *et al*., 2002; Bahadori *et al*., 2004; Arabali & Hooshyar, 2009) which reported no effect of sex on the intensity of infection.

However, with regard to age, older animals are more prone to acquire the infection. The age of the cat was found to be an important risk factor associated with parasitic infection, with immature cats being more likely to be parasitized than adult cats. These findings are similar to those obtained in previous studies (Visco *et al*., 1978; Wilson-Hanson & Prescott, 1982; Shaw *et al*., 1983; Nolan & Smith, 1995; Hill *et al*., 2000; Spain *et al*., 2001; Barutzki & Schaper, 2003; Palmer *et al*., 2008; Gates & Nolan, 2009; Mircean *et al*., 2010). It is probable that infection can occur at any age, either by eggs or tissue containing the larvae, although the highest incidence of infection occurs in immature cats.

CONCLUSIONS

High prevalence rate of stray cats with *T. cati* parasite in the studied area suggests that inhabitants face risk of *T. cati* parasitic infections through contact with infected cats and their excretion. The highest incidence of infection occurs in immature cats and sex seemed to have no effect on prevalence of parasitism. It is clear that the worldwide distribution of stray cats would have an impact on sanitation. Therefore, both animal and human health education are recommended and there is a need to plan adequate control programs to diagnose, treat and control of *T. cati* parasite in stray cats in the region.

Conflict of interest statement: The authors declare no conflict of interest.

REFERENCES

Arabali, M. & Hooshyar, H. (2009). Gastrointestinal parasites of stray cats in Kashan, Iran. *Tropical Biomedicine* **26(1)**: 16-22.

Bahadori, S.H.R., Eslami, A., Meshgi, B. & Poor, H.S. (2004). Study on stray cats infected with parasitic helminthes in Tehran. *Journal of Veterinary Faculty, University of Tehran* **59(2)**: 171-174.

Barutzki, D. & Schaper, R. (2003). Endoparasites in dogs and cats in Germany 1999-2002. *Parasitology Research* **90**: S148-S150.

Borecka, A. & Gawor, J. (2008). Modification of gDNA extraction from soil for PCR designed for the routine examination of soil samples contaminated with *Toxocara* spp. eggs. *Journal of helminthology* **82**: 119-122.

Calvete, C., Lucientes, J., Castillo, J.A., Estrada, R., Garcia, M.J. & *et al*. (1998). Gastrointestinal helminth parasites in stray cats from the mid-Ebro Valley, Spain. *Veterinary Parasitology* **75**: 235-240.

Dalimi, A.H. & Mobedi, I. (1992). Helminthes parasites of carnivores in northern Iran. *Annals of Tropical Medicine and Parasitology* **68**: 395-397.

Dubinsky, P. (1999). New approaches to control larval Toxocariosis. *Helminthology* **6**: 159-165.

Fisher, M. (2003). *Toxocara cati*: an underestimated zoonotic agent. *Trends in Parasitology* **19(4)**: 167-170.

Gates, M.C. & Nolan, T.J. (2009). Endoparasite prevalence and recurrence across different age groups of dogs and cats. *Veterinary Parasitology* **166**: 153-158.

Glickman, L.T. & Schantz, P.M. (1981). Epidemiology and pathogenesis of zoonotic toxocariasis. *Epidemiologic Reviews* **3**: 230-250.

Haralampidis, S.T. (1977). Simbole sti melete ton parasitin tez gatas. Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Greece: Aristotle University of Thessaloniki.

Hill, S.L., Cheney, J.M., Taton-Allen, G.F., Reif, J.S., Bruns, C. & Lappin, M.R. (2000). Prevalence of enteric zoonotic organisms in cats. *Journal of the American Veterinary Medical Association* **216**: 687-692.

Hosseini, S.R., Sekhavatmandi, A. & Khamesipour, F. (2017). PCR based analysis of Haemobartonellosis (Candidatus mycoplasma haematoparvum and Mycoplacma haemocanis) and its prevalence in dogs in Isfahan, Iran. *Bioscience Biotechnology Research Communications* **10(2)**: 187-191.

Jamshidi, S.H., Meshgi, B. & Toghani, M. (2002). A study of helminthic infection of gastrointestinal tract in stray cats at urban, area in Isfahan. *Journal of Veterinary Faculty, University of Tehran* **57(2)**: 25-27.

Khademvatan, S., Rahim, F., Tavalla, M., Abdizadeh, R. & Hashemitabar, M. (2013). PCR-Based Molecular Characterization of *Toxocara* spp. Using Feces of Stray Cats: A Study from Southwest Iran. Brayton KA, ed. *PLoS ONE* **8(6)**: e65293.

Khamesipour, F., Doosti, A., Iranpour Mobarakeh, H. & Komba, E.V.G. (2014a). *Toxoplasma gondii* in Cattle, Camels and Sheep in Isfahan and Chaharmahal va Bakhtiary Provinces, Iran. Jundishapur *Journal of Microbiology* **7(6)**: e17460. http://doi.org/10.5812/jjm.17460.

Khamesipour, F., Doosti, A., Koohi, A. & Radojicic, B. (2014b). Molecular survey for the presence of Corynebacterium pseudotuberculosis from sheep and camel lymph nodes by PCR in Iran. *Journal of Camel Practice and Research* **21(2)**: 205- 208.

Kreceka, R.C., Mouraa, L., Lucasa, H. & Kellya, P. (2010). Parasites of stray cats (Felis domesticusL., 1758) on St. Kitts, West Indies. *Veterinary Parasitology* **172**: 147-149.

Lee, A.C., Schantz, P.M., Kazacos, K.R., Montgomery, S.P. & Bowman, D.D. (2010). Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. *Trends in Parasitology* **26(4)**: 155-161.

Macpherson, C.N. (2013). The epidemiology and public health importance of toxocariasis: a zoonosis of global importance. *International Journal for Parasitology* **43(12-13)**: 999-1008.

Malloy, W.F. & Embil, J.A. (1978). Prevalence of *Toxocara* spp. and other parasites in dogs and cats in Halifax, Nova Scotia. *Canadian Journal of Comparative Medicine* **42**: 29-31.

McColm, A.A. & Hutchison, W.M. (1980). The prevalence of intestinal helminths in stray cats in central Scotland. *Journal of Helminthology* **54**: 255-257.

Mircean, V., Titilincu, A. & Vasile, C. (2010). Prevalence of endoparasites in household cat (Felis catus) populations from Transylvania (Romania) and association with risk factors. *Veterinary Parasitology* **171**: 163-166.

Mizgajska-Wiktor, H. & Uga, S. (2006). *Toxocara cati* eggs in the environmental contamination. Exposure and environmental contamination. In: Toxocara: the enigmatic parasite. (Editors, C.V. Holland & H.V. Smith), 211-227.

Nekoei, S., Taktaz Hafshejani, T., Doosti, A. & Khamesipour, F. (2015). Molecular detection of bovine leukemia virus in peripheral blood of Iranian cattle, camel and sheep. *Polish Journal of Veterinary Sciences* **18(4)**: 703-707.

Niak, A. (1972). The prevalence of *Toxocara cati* and other parasites in Liverpool cats. *Veterinary Record* **91**: 534-536.

Nichol, S., Ball, S.J. & Snow, K.R. (1981). Prevalence of intestinal parasites in feral cats in some urban areas of

England. *Veterinary Parasitology* **9**: 107-110.

Nolan, T.J. & Smith, G. (1995). Time series analysis of the prevalence of endoparasitic infections in cats and dogs presented to a veterinary teaching hospital. *Veterinary Parasitology* **59**: 87-96.

Overgaauw, P.A.M. (1997). Aspects of toxocara epidemiology: toxocarosis in dogs and cats. *Critical Reviews in Microbiology* **23**: 233-251.

Overgaauw, P.A. (1997). Prevalence of intestinal nematodes of dogs and cats in the Netherland. *The Veterinary Quarterly* **19**: 14-17.

Palmer, C.S., Thompson, R.C., Traub, R.J., Rees, R. & Robertson, I.D. (2008). National study of the gastrointestinal parasites of dogs and cats in Australia. *Veterinary Parasitology* **151**: 181-190.

Rai, S.K., Uga, S., Ono, K., Rai, G. & Matsumura, T. (2000). Contamination of soil with helminth parasite eggs in Nepal. *The Southeast Asian Journal of Tropical Medicine and Public Health* **31(2)**: 388-93.

Robertson, I.D. & Thompson, R.C. (2002). Enteric parasitic zoonoses of domesticated dogs and cats. *Microbes and Infection* **4**: 867-873.

Shakerian, A., Deo, P., Rahimi, E., Shahjavan, A.R. & Khamesipour, F. (2016). Molecular detection of Brucella melitensis in sheep and goat milk in Iran. *Tropical Journal of Pharmaceutical Research* **15(5)**: 913-918.

Shaw, J., Dunsmore, J. & Jakob-Hoff, H.R. (1983). Prevalence of some gastrointestinal parasites in cats in the Perth area. *Australian Veterinary Journal* **60**: 151-152.

Sommerfelt, L.E., Cardillo, N., Lopez, C., Ribicich, M., Gallo, C. & Franco, A. (2006). Prevalence of *Toxocara cati* and other parasites in cats' faeces collected from the open spaces of public institutions: Buenos Aires, Argentina. *Veterinary Parasitology* **140**: 296-301.

Spain, C.V., Scarlett, J.M., Wade, S.E. & McDonough, P. (2001). Prevalence of enteric zoonotic agents in cats less than1 year old in Central New York State. *Journal of Veterinary Internal Medicine* **15**: 33-38.

Tajbakhsh, E., Ahmadi, P., Abedpour-Dehkordi, E., Arbab-Soleimani, N. & Khamesipour, F. (2016). Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic *E. coli* isolated from clinical samples in Iran. *Antimicrobial Resistance and Infection Control* **5**: 11. http://doi.org/10.1186/s13756-016-0109-4.

Talvik, H., Moks, E., Magi, E., Jarvis, T. & Miller, I. (2006). Distribution of Toxocara infection in the environment and in definitive and paratenic hosts in Estonia. *Acta Veterinaria Hungarica* **54**: 399-406.

Visco, R.J., Corwin, R.M. & Selby, L.A. (1978). Effect of age and sex on the prevalence of intestinal parasitism in cats. *Journal of the American Veterinary Medical Association* **172(7)**: 797-800.

Wilson-Hanson, S.L. & Prescott, C.W. (1982). A survey for parasites in cats. *Australian Veterinary Journal* **59**: 194.

Yaman, M., Ayaz, E., Gul, A. & Muz, M.N. (2006). Investigation of helminth infections of cats and dogs in the Hatay province. *Türkiye Parazitoloji Derneði* **30**: 200-204.

Yang, Y. & Liang, H. (2015). Prevalence and Risk Factors of Intestinal Parasites in Cats from China. *BioMed Research International* **2015**: Article ID 967238, 5 pages. doi:10.1155/2015/967238.

Zibaei, M., Sadjjadi, S.M. & Sarkari, B. (2007). Prevalence of *Toxocara cati* and other intestinal helminths in stray cats in Shiraz, Iran. *Tropical Biomedicine* **24(2)**: 39-43.